Monkeypox-specific antibodies in human and simian sera from the Ivory Coast and Nigeria*

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*A test for monkeypox-specific antibodies is described. Monkeypox immune sera can be made type-specific by immunoabsorption with heterotypic poxvirus extracts. Monkeypox-specific antibodies were demonstrated in sera from 9 cynomolgus monkeys (*Macaca fascicularis*) that had previously been experimentally infected with monkeypox. Monkeypox-specific antibodies were found in 3 wild-caught African monkeys (*Cercopithecus *spp.*) and in 3 human sera collected from Africans in the Ivory Coast and Nigeria 3½–4 years after they had suffered a pox-like infection. Monkeypox had been recognized in one of the patients by virus isolation, and had been suspected in the others for epidemiological reasons. Vaccinia-specific antibodies were found in 4 human sera collected 6 weeks after smallpox vaccination.*

The serological results provide the first laboratory evidence of a monkeypox reservoir in wild monkeys.

Monkeypox has appeared in spontaneous outbreaks in captive monkey colonies (*2*, *8*) and human monkeypox infections have occurred in smallpox free areas of West and Central Africa (*3*). In humans the disease cannot be distinguished clinically from smallpox without isolation and characterization of the virus.

Monkeypox virus isolates from wild monkey populations have not been reported. A serological survey of monkeys from various continents, with a view to tracing foci of infection, was unsuccessful (*1*).

Variola can be transmitted to cynomolgus monkeys (*Macaca fascicularis*) experimentally by aerosol and by contact and the infection can be serially passaged in these animals for not more than 2–6 generations (*10*). Marennikova et al. (*9*) obtained a poxvirus isolate from the kidneys of a wild-caught African monkey. It differed from monkeypox but could not be distinguished from variola virus. A similar virus had been obtained previously from healthy cynomolgus monkey kidneys (*4*). The name " white poxvirus " has been proposed for this virus because of the white lesions it produces on the chick chorioallantoic membrane (CAM). White poxvirus differs essentially from white mutants of monkeypox virus which also produce white lesions on CAM. The white mutants resemble the parental monkeypox virus in all respects except for their white lesions in egg membranes (*4*).

Three specific antigens have been described recently: *va*, *mo*, and *vc*. These antigens correspond to three serotypes that can be distinguished with specific antisera in double immunodiffusion tests (*5*).

The present paper is a report on the typing of poxvirus antibodies in sera from patients and monkeys that had been immunized naturally or by experimental infection.

MATERIAL AND METHODS

Sera

Nine cynomolgus monkeys (*Macaca fascicularis*) were infected with monkeypox virus by scarification. Sera were obtained from the monkeys after intervals from 2 months up to 5 years. Sera were also obtained from a cynomolgus monkey that had been infected by variola (Tilburg) and from another that had been infected with a variola-like white poxvirus (chimp 9). The latter had been isolated by Marennikova et al., (*9*) and was received through the intermediary of the World Health Organization.

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Sera of 13 wild-caught monkeys (*Cercopithecus* spp.) from the Ivory Coast, were received from Dr J. H. Nakano for screening for poxvirus antibodies.

Sera from cases of proved or presumed human monkeypox infection were collected by Dr R. Netter from 2 individuals in Nigeria and 1 in the Ivory Coast who had a history of a pox-like disease. Samples of the sera, coded A. K., Mrs K. and Y., were obtained through the intermediary of the World Health Organization.

Sera were also obtained from 4 individuals who had been inoculated with smallpox vaccine 6 weeks previously.

Virus isolates

Monkeypox isolate, Utrecht (65-32), was derived from an orang-utan during an outbreak in the Rotterdam Zoo (*7*). Monkeypox isolate, Copenhagen (*8*), was used as a prototype. " White poxvirus " isolate, chimp 9, was isolated from a wild chimpanzee caught in Zaire (*9*). The virus could not be distinguished by laboratory methods from variola virus.

Vaccinia virus, Denmark, was derived from a smallpox vaccine sample that had been prepared with a vaccinia virus strain originally obtained from the State Serum Institute, Copenhagen.

Neutralization (N) and immunofluorescence (IF) tests for poxvirus antibodies

Persistent poxvirus antibodies were titrated by a neutralization method based on 50% pock count reduction and by indirect immunofluorescence. Vaccinia- and monkeypox-infected BHK cells were used for preparing IF antigens. Variola antigens were not used as they were less suitable.

Both tests were performed as described previously by Gispen et al. (*6*).

Immunofluorescence test for type-specific antibodies

The test differed from the indirect immunofluorescence test for poxvirus antibodies merely in its absorption procedure for test sera, which was supplemented by immunoabsorption.

Chorioallantoic membranes with confluent lesions of vaccinia Denmark or monkeypox Copenhagen were homogenized in a precooled (−25°C) mortar and then suspended in 1.0 ml of distilled water per membrane. The suspension was centrifuged at 800 *g* for 10 minutes. The supernatant was freeze-dried in ampoules containing 0.5 ml of the suspension. The dried suspension was stored at 4°C and used as the immunoabsorbent.

Immunoabsorption was combined with the absorption of non-specific fluorescent matter. 0.1 ml of test serum and 0.3 ml of a 20% hamster brain suspension in egg yolk were pipetted on to the dried immunoabsorbent in the ampoule. The mixture was homogenized by shaking and kept at 37°C for 2 hours and at 4°C overnight. The mixture was centrifuged at 36 200 *g* for 2 hours. When necessary the supernatant was added to a fresh portion of the dried immunoabsorbent to repeat the procedure. Sometimes three successive immunoabsorptions were required to remove all the antibody. The supernatant of the last immunoabsorption procedure was pipetted on to 100 mg of wetted hamster kidney powder in a centrifuge tube. The tube was shaken and then placed at 4°C for 1 hour. The tube was centrifuged at 800 *g* for 2 hours. The supernatant was diluted with an equal volume of barbital buffered saline, pH 7.2, containing 10% fresh guinea-pig serum. This corresponded to a 1:8 dilution of the test serum. It was used for the indirect IF test with vaccinia and monkeypox antigen preparations.

RESULTS

Experimentally infected monkeys

The sera were obtained from 9 cynomolgus monkeys that had been infected by scarification with monkeypox several years previously and kept in our laboratory with a view to studying persistent antibodies. Two aliquots of each serum were treated with vaccinia and monkeypox immunoabsorbents, respectively. Both of the absorbed parts and the unabsorbed serum were tested by indirect immunofluorescence with vaccinia and monkeypox antigens.

The unabsorbed monkeypox sera showed positive reactions with both vaccinia and monkeypox antigens. All antibodies could be absorbed by monkeypox extracts. Vaccinia absorbent removed part of the antibodies only. The monkeypox-specific antibodies were not removed and reacted by immunofluorescence in dilutions from 64 up to 256 (Table 1). On the contrary, serum of monkey No. 11, with a history of an experimental white poxvirus infection, showed a vaccinia-specific IF reaction. Serum of monkey No. 12, with a history of smallpox infection, was negative for antibodies of both specificities.

Four of the vaccinia-absorbed sera (monkeys 5, 6, 7, and 24) with comparatively high IF titres for

Table 1. Monkeypox-specific antibodies after experimental infection in cynomolgus monkeys. Pre-infection sera: antibody negative

Monkey No.	Infection with isolate [a]	Interval since infection (years)	Test serum absorbed with	Immunofluorescence titre with antigens of	
				monkeypox [b]	vaccinia [b]
1	MP Utrecht	5	unabsorbed	256	512
			monkeypox	–	–
			vaccinia	128	–
3	MP Copenhagen	2	unabsorbed	512	512
			monkeypox	–	–
			vaccinia	128	–
4	MP Copenhagen	3	unabsorbed	512	512
			monkeypox	–	–
			vaccinia	64	–
5	MP Copenhagen	3	unabsorbed	2048	2048
			monkeypox	–	–
			vaccinia	256	–
6	MP Copenhagen	3	unabsorbed	512	1024
			monkeypox	–	–
			vaccinia	128	–
7	MP Copenhagen	2	unabsorbed	1024	512
			monkeypox	–	–
			vaccinia	128	–
22	MP Utrecht	2 months	unabsorbed	1024	1024
			monkeypox	–	–
			vaccinia	64	–
23	MP Utrecht	2	unabsorbed	1024	1024
			monkeypox	–	–
			vaccinia	64	–
24	MP Utrecht	2	unabsorbed	2048	2048
			monkeypox	–	–
			vaccinia	256	–
11	White poxvirus (Chimp 9)	3	unabsorbed	2048	2048
			monkeypox	–	128
			vaccina	–	–
12	Variola (Tilburg)	3	unabsorbed	512	512
			monkeypox	–	–
			vaccinia	–	–

[a] MP = monkeypox.
[b] A dash indicates that the titre was less than 8.

monkeypox-specific antibodies, were tested by immunodiffusion. No precipitation line was visible.

Specific antibodies after naturally acquired infections

Serum samples of 13 wild-caught African monkeys were examined for poxvirus antibodies by neutralization and immunofluorescence tests. Four of the samples were positive by both methods and were accepted as antibody-containing sera (Table 2).

The 4 positive samples were tested for specific antibodies. Two of the sera, 151-32 and 151-5, after absorption reacted as monkeypox specific in dilutions up to 32 and 16, respectively. One serum, 151-42, also showed a monkeypox-specific reaction in dilutions up to 16, but too little serum was available to demonstrate whether the specific antibodies could be removed by monkeypox absorbent. The fourth sample, 151-40, showed common but no specific antibody reaction (Table 3).

Sera of 2 inhabitants of Nigeria (A. K. and her mother, Mrs K.) and one of Ivory Coast (Y.) were examined for monkeypox-specific antibodies. The 3 donors had suffered from a pox-like disease 3½–4 years previously. The infection of A. K. had been recognized as monkeypox by virus isolation (CDC, Atlanta). Mrs K. felt ill 11 days later than

Table 2. Sera of 13 African monkeys tested for poxvirus antibodies

Cercopithecus species	Serum No.	Antibody titre	
		IF [a]	Neutralization [a]
C. aethiops	151–32	1024	29
C. aethiops	151– 5	256	115
C. petaurista	151–42	256	102
C. mona	151–46	–	–
C. aethiops	151–14	–	26
C. mona	151–16	–	–
C. aethiops	151–24	–	–
C. aethiops	151–40	128	100
C. aethiops	211– 4	–	–
C. aethiops	211– 7	–	–
C. aethiops	211–15	–	–
C. aethiops	211–22	–	–
C. aethiops	211–34	–	–

[a] A dash indicates that the titre was less than 4.

her daughter and might have been infected by contact with the index case. Donor Y. had suffered from a solitary pox-like infection while living in a smallpox-free area. The pox-like character of his disease was confirmed by a serological response demonstrated in paired sera by complement fixation (Center for Disease Control, Atlanta, GA, USA).

Absorption of the 3 serum samples with vaccinia antigen removed all antibodies for vaccinia but not those for monkeypox. Monkeypox-specific antibodies could be demonstrated after absorption in each of the three sera in dilutions ranging from 16 to 64 (Table 3).

Sera were obtained from 4 Europeans who had never been in tropical areas and had been vaccinated against smallpox 6 weeks previously. The sera served as controls in the specific antibody test. None of the 4 sera showed monkeypox-specific antibodies, but all had vaccinia specificity (Table 3).

DISCUSSION

Three serotypes of orthopoxviruses have been defined which correspond to the production of one

Table 3. Naturally acquired monkeypox-specific antibodies in sera from wild caught African monkeys and in human sera that were collected 3½–4 years after proved or presumed monkeypox. Controls: human sera after smallpox vaccination.

Code	Origin of sera	Interval since infection	Immunofluorescence titre of		
			Poxvirus antibodies (vaccinia)	Type-specific antibodies for antigens of:	
				monkeypox [a]	vaccinia [a]
151–32	*C. aethiops*		1024	32	–
151– 5	*C. aethiops*		512	16	–
151–42	*C. petaurista*		256	16 [b]	–
151–40	*C. aethiops*		64	–	–
A. K.	proved monkeypox	4 years	128	16	–
Mrs K.	infected contact of A. K.	4 years	256	64 [b]	–
Y.	presumed monkeypox	3½ years	256	32	–
38	control after vaccination	6 weeks	2048	–	64
173	control after vaccination	6 weeks	512	–	128
207	control after vaccination	6 weeks	512	–	64
225	control after vaccination	6 weeks	1024	–	64

[a] A dash indicates that the titre was less than 8.
[b] Too little serum was available for testing with monkeypox absorbent.

or more of three specific antigens: *va* in variola, *mo* in monkeypox and *vc*+*va* in vaccinia infections (*5*). Typing of poxvirus isolates by double immunodiffusion requires highly potent immune rabbit sera that have been made type-specific by absorption with heterotypic virus extracts. The lower concentration of antibodies may be the reason why monkey sera acquired after natural or experimental infection are unsatisfactory in type-specific immunodiffusion tests.

The need for a suitable method for antibody typing became urgent when Dr Nakano made available positive sera from wild monkeys caught in the Ivory Coast. As monkeypox infection had never been demonstrated in free-living monkeys it seemed worth while to test the antibodies in these animals for monkeypox- and vaccinia-specificity. The method described appeared satisfactory for typing monkeypox-specific antibodies induced by infection in monkeys and man. It was possible to distinguish these sera from sera from 4 Europeans collected 6 weeks after smallpox vaccination. It should be remembered here that monkeypox-specificity in IF tests cannot simply be identified with *mo*-specificity as shown in double immunodiffusion tests.

Monkey sera in which antibodies have been induced by infection with variola or white poxvirus should be investigated further. Variola (vaccinia)-specific antibodies have been obtained with both viruses in rabbits (*5*). Similar specific antibodies were repeatedly found in monkeys after infection with white poxvirus chimp 9, but not after infection with variola Tilburg virus in monkeys (Table 1). The different specific antibody responses in monkeys shown by serologically related isolates may be explained by virus–host interactions, which may vary quantitatively between isolates as well as between hosts.

There seems to be little doubt that 3 of the 4 wild caught African monkeys had antibodies that had been induced by monkeypox infection. The fourth monkey showed a 4–16 times lower serum titre for poxvirus antibodies than the other three. This may explain why neither monkeypox- nor vaccinia-specific antibodies could be demonstrated in this monkey serum.

The results suggest that human monkeypox infections, as reported from Africa, may be explained by contact with a virus reservoir in the wild monkey population.

The test for monkeypox-specific antibodies makes possible a serological diagnosis in cases of presumed monkeypox when virus has not been isolated. It may also be a useful tool for surveillance in smallpox-free tropical countries.

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RÉSUMÉ

ANTICORPS SPÉCIFIQUES DU MONKEYPOX DANS DES SÉRUMS HUMAINS ET SIMIENS RECUEILLIS EN CÔTE D'IVOIRE ET AU NIGÉRIA

Une épreuve pour l'identification des anticorps spécifiques du monkeypox est décrite dans le présent article. Des immunsérums anti-monkeypox peuvent être rendus spécifiques de type par immuno-absorption d'extraits de poxvirus hétérotypiques. Des anticorps spécifiques du monkeypox ont été mis en évidence dans les sérums de 9 singes cynomolgus (*Macaca fascicularis*) qui avaient été quelque temps auparavant infectés expérimentalement avec des virus monkeypox. Des anticorps spécifiques du monkeypox ont été également observés chez trois singes africains (*Cercopithecus* spp.) capturés à l'état sauvage et dans trois sérums humains recueillis en Côte d'Ivoire et au Nigéria chez des Africains qui avaient souffert d'une infection de type pox 3½–4 ans auparavant. Le monkeypox avait été reconnu chez l'un des malades par isolement du virus et soupçonné chez les autres pour des raisons épidémiologiques. Des anticorps spécifiques de la vaccine ont été trouvés dans quatre sérums humains prélevés six semaines après la vaccination antivariolique.

Ces résultats sérologiques constituent la première preuve obtenue en laboratoire de l'existence d'un réservoir de monkeypox chez les singes sauvages.

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